American Journal of Hematology 61:26–33 (1999)

Restricted Diversification of T-Cells in Chronic Active Epstein-Barr Virus Infection: Potential Inclination to T-Lymphoproliferative Disease

Shouichi Ohga,[1]* Nobuhiro Kimura,[2] Hidetoshi Takada,[1] Mituyuki Nagano,[2] Kohichi Ohshima,[3] Akihiko Nomura,[1,4] Kenji Muraoka,[4] Hiromichi Take,[4] Shunji Yamamori,[5] and Toshiro Hara[1]

[1]Department of Pediatrics, Faculty of Medicine, Kyushu University, Fukuoka, Japan
[2]The First Department of Internal Medicine, School of Medicine, Fukuoka University, Fukuoka, Japan
[3]The First Department of Pathology, School of Medicine, Fukuoka University, Fukuoka, Japan
[4]Division of Pediatrics, Kagoshima Municipal Hospital, Kagoshima, Japan
[5]Division of Gene Analysis, Mitsubishi Bio-Chemical Laboratories, Inc., Tokyo, Japan

To assess the abnormal T-cell expansion in chronic active Epstein-Barr virus infection (CAEBV), T-cell antigen receptor (TCR) repertoire was analyzed in four patients with the disease. All fulfilled the diagnostic criteria of CAEBV, presenting with fever, hepatosplenomegaly, cytopenia, abnormal high titers of anti EBV-antibodies, and positive EBV genome of unknown cause. Southern blotting probed with EBV-terminal repeats and TCR Cβ gene indicated clonal expansion of the infected cells in 3 and 2 patients, respectively. The number of $CD4^+$ $HLA\text{-}DR^+$ cells appreciably increased in patients 1 (59%) and 2 (24%), who had a coronary aneurysm and central nervous system involvement, respectively. TCR gene expression examined by the inverse polymerase chain reaction methods revealed that Vβ gene usages were preferential in all patients (Vβ7 and Vβ12: patient 1, Vβ4: patient 2, Vβ13: patients 3 and 4), compared with those in healthy controls. Vα18 gene expression was remarkably high in patients 1 and 2. Moreover, Jβ gene expression was skewing in the reigning Vβ clones in all patients. Vβ4-Jβ1.5 and Vβ13-Jβ1.5 genes were clonally expressed in patients 2 and 4, respectively. These results suggest that CAEBV is associated with the restricted diversity of T-cells, which may stem from the sustained expansion of oligoclonal T-cells possibly driven by conventional viral antigens, but not, superantigens. Although the study is limited by the small number of patients, the unbalanced T-cell repertoire might contribute to the evolution of T-lymphoproliferative disease, otherwise, imply the innate defective immunity to EBV in CAEBV patients. Am. J. Hematol. 61:26–33, 1999.

Key words: CAEBV; EBV genome; LPD; TCR repertoire

INTRODUCTION

Primary infection of Epstein-Barr virus (EBV) leads to subclinical or acute infectious mononucleosis (IM) in susceptible immunocompetent subjects. EBV is incriminated as a causative agent in an array of malignancies including Burkitt's lymphoma, Hodgkin's or non-Hodgkin's lymphoma, nasal T-cell lymphoma, pyothorax-associated lymphoma and nasopharyngeal, thymic, or gastric carcinoma [1–6]. Primary and secondary immunodeficiencies occasionally elicit the persistent reactivation of EBV and the subsequent development of EBV-associated lymphoproliferative disease (LPD), most of which originate from the outgrowth of EBV-infected B lymphocytes [7–10]. The clinical entity of chronic active EBV infection (CAEBV) has been established as a primary persistent mononucleosis character-

Contract grant sponsor: Ministry of Health and Welfare for Primary Immunodeficiency Diseases; Contract grant sponsor: Ministry of Education, Science and Culture of Japan.

*Correspondence to: Shouichi Ohga, M.D., Department of Pediatrics, Faculty of Medicine, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan. E-mail: ohgas@mailserver.med.kyushu-u.ac.jp

Received for publication 27 July 1998; Accepted 6 January 1999

ized by fever, cytopenia, hepatosplenomegaly along with the clonal proliferation of EBV [11–14]. This disease is supposed to render the defective T-cell immunity to EBV and develop the lymphoid malignancy [15,16]. There has been convincing evidence that EBV can infect and proliferate T-cells and natural killer (NK) cells as well as B-cells [17–22]. It may be associated with the evolution of clonal lymphoproliferation and/or lymphomas of T/NK-cells; however, the pathogenesis has not been elucidated.

In this study, we investigated the T-cell repertoire in CAEBV patients by using the novel inverse polymerase chain reaction (PCR) methods [23], which could allow us to perform a quantitative analysis on the usages of T-cell antigen receptor (TCR) variable (V)/joining (J) region genes in lymphocytes. The possible association between the aberrant T-cell expansion and the clonal evolution in CAEBV is then discussed.

PATIENTS and METHODS

Clinical Profiles of Patients

Clinical characteristics of four Japanese children (3 males and 1 female) with CAEBV are shown in Table I. Each patient fulfilled the diagnostic criteria of the disease [11,12]. There was no consanguineous marriage, or immunohematologic disorders in their families. None of them had mosquito allergy [22]. Patient 1, a 6-year-old boy first presented with splenomegaly and periodic high fever [24]. This patient deteriorated with the development of coronary artery aneurysm, which subsided by interferon (IFN)-α therapy. Peripheral blood was obtained for this study before the administration of IFN-α. Patient 2 was diagnosed with CAEBV at 14 months of age. Various noncytotoxic agents could not confer the prolonged resolution. At 8 years of age, he was splenectomized for the disease progression accompanied by AV block and retinopathy. Following 2 years of clinical remission, central nervous system (CNS)-LPD arose. Brain biopsy disclosed the infiltration of $CD4^+$, $CD45RO^+$, and EBV-encoded RNA (EBER) 1^+ T-cells. Blood sampling was performed at the time of the diagnosis of CNS-LPD. Patients 3 and 4 were diagnosed with CAEBV at 14 and 12 years of age, respectively. They showed hepatitis and splenomegaly, but required no intensive treatment for more than 2 years after the onset of disease.

TABLE I. Characteristics of Patients With Chronic Active Epstein-Barr Virus Infection*

Patients	1	2	3	4
Age (years)	8	11	16	14
Age of onset (years)	6	1y 2m	9	12
Sex	M	M	F	M
Clinical findings				
Fever	Yes	Yes	Yes	Yes
Cytopenia	Yes	Yes	Yes	Yes
Hepatosplenomegaly	Yes	Yes	Yes	Yes
Arrhythmia	No	Yes	No	No
Coronary lesion[a]	Yes	No	No	No
Ocular involvement	No	Yes	No	No
CNS lesion[a]	No	Yes	No	No
Laboratory findings				
Impaired PHA response	No	Yes	No	No
Depressed NK activity	Yes	Yes	No	No
VCA-IgG	1,280	10,240	10,240	5,120
IgA	10	160	320	160
IgM	<10	<10	<10	<10
EADR-IgG	1,280	640	640	1,280
IgA	10	<10	20	40
EBNA	10	160	10	40
$CD3^+HLA\text{-}DR^+$ (%)	60	49	19	20
$CD4^+CD8^-$ (%)	59	24	14	4
$CD4^-CD8^+T$ (%)	6	14	7	16
CD4/CD8	4.4	1.9	1.5	0.7
EBV clonality[b]	Mono	Poly	Mono	Mono
TCR rearrangement[b]	R	G	R	G
Ig rearrangement[b]	G	NT	G	G

*M, male; F, female; EBV, Epstein-Barr virus; TCR, T-cell antigen receptor; Ig, immunoglobulin; Mono, monoclonal band; Poly, polyclonal smear; R, rearrangement band; G, germline band; NT, not tested.

[a]Blood sampling was performed before the development of coronary lesion in patient 1, and at the diagnosis of central nervous sytem (CNS)-lymphoproliferative disease in patient 2.

[b]EBV clonality and TCR/Ig gene rearrangement were assessed by Southern blotting.

Surface Marker Analysis

Peripheral blood was obtained from four CAEBV patients. Informed consent was obtained from each patient and/or their guardians for this study. Flow cytometric analysis was performed using an EPICS XL (Coulter, Miami, FL) as described previously [25]. The forward light scatter gate was set to analyze viable lymphocytes and exclude background artifacts. Multi-color staining was carried out using fluorescein isothiocyanate (FITC)-, phycoerythrin (PE)-, or phycoerythrin-cyanin 5.1 (PECy5)-conjugated monoclonal antibodies (Abs) of TCR1αβ (Becton Dickinson, San Jose, CA), CD3, CD4, HLA-DR (Becton Dickinson; Immunotech Coulter, Miami, FL), CD8, CD19, CD56 (Immunotech Coulter).

Detection of EBV Genome

High molecular DNA was extracted from peripheral mononuclear cells (PMC), bone marrow (BM), or spleen cells. Dot blotting to detect the EBV genome was performed based on the conventional PCR protocol, using the EBV primer specific for a 240 base pair (bp) region within the *Bam*HI-K region containing the internal repeated (IR) sequences of the EBV genome as described previously [19]. In this PCR protocol, DNA extracted from a cell line (Raji) or the PMC of a patient with acute IM shows positive, and DNA from the PMC of healthy subjects who had no antibodies to EBNA shows negative EBV genome.

Southern Blot Analysis Probed With EBV-Terminal Repeats or TCR/Ig Genes

Southern blotting was performed as described previously [19]. Briefly, 5 μg of high molecular weight DNA were digested with *Eco*RI, *Bam*HI, *Hind*III, and/or *Kpn*I. The digested DNA was electrophoresed on 0.9% agarose gel and transferred to Byodine-B, which was hybridized with a ^{32}P-labeled probe. Clonality of lymphocytes was assessed using the probes of a 5.2-kb *Bam*HI-*Eco*RI fragment containing the tandem terminal repeated (TR) sequence of the EBV genome, and/or the TCR/immunoglobulin (Ig) gene probes of Cβ1, Jβ1, Jβ2, Cγ, IgH, κ, and λ.

Inverse PCR Method for Analyzing TCR Vα,Vβ, and Jβ Gene Usages

The newly devised inverse PCR method was applied to analyze the TCR repertoire [23]. Briefly, oligo(dT)-primed double stranded cDNA was synthesized from 1 μg of total RNA using reverse transcriptase, RNase H, *Escherichia coli* (*E. coli*) DNA polymerase I, and *E. coli* DNA ligase, followed by the incubation with T4 DNA polymerase for blunt-end formation. Following the construction of circular double stranded cDNA by T4 DNA ligase, PCR was performed separately using two constant region primers of Cα or Cβ that are in opposite directions: Cα (forward), 5′-GGG TCG ACG ACC TCA TGT CTA GCA CAG T-3′; and Cα (inverse) , 5′-GCA TGC GGC CGC CCT GCT ATG CTG TGT GTC T-3′; or Cβ (forward), 5′-GGG TCG ACC TGT GCA CCT CCT TCC CAT T-3′, and Cβ (inverse), 5′-GCA TGC GGC CGC ATG GCC ATG GTC AAG AGA-3′. After 30 cycles of PCR (denaturation at 95°C for 30 sec, annealing at 62°C for 30 sec, and extension at 72°C for 1 min), the amplified PCR mixture was electrophoresed on 1.5% low-melting-point agarose, and then the cDNA fragments expected sizes between 600 bp and 950 bp were extracted to be used as a probe. The cDNA fragments were labeled with α-^{32}P-dCTP and hybridized to the filters, on which 200 ng of each Vβ fragment (Vβ1≈Vβ20) or Vα fragment (Vα1≈Vα18, Vα21, Vα24) were dotted. Each V-specific fragment size ranging from 280 bp to 350 bp was prepared from the series of HBVT/HBVP or HAVT/HAVP plasmids originated from thymus or peripheral T-cell [26]. Since this PCR protocol employs two opposite directional C region gene primers to be hybridized with each V region gene, the spot intensity represents the V gene usages within the equally amplified TCR clones. Using the densitometry, the quantitative assessment of V gene usage was made from the ratio (%) of the total amounts of hybridized products on each V family gene. When a certain Vβ gene was preferentially used, Jβ gene usages in the corresponding Vβ clone were also examined. RT-PCR amplification of the RNA was performed using the sense Vβ gene primer and a total of 13 sets of antisense Jβ primers. The antisense Jβ primers were as follows: Jβ1.1, 5′-AAC TGT GAG TCT GGT GCC TTG-3′; Jβ1.2, 5′-AAC GGT TAA CCT GGT CCC CGA-3′; Jβ1.3, AAC AGT GAG CCA ACT TCC CTC-3′; Jβ1.4, 5′-GAC AGA GAG CTG GGT TCC ACT-5′; Jβ1.5, 5′-GAT GGA GAG TCG AGT CCC ATC-3′; Jβ1.6, 5′-CAC AGT GAG CCT GGT CCC ATT-3′; Jβ2.1, 5′-CAC GGT GAG CCG TGT CCC TGG-3′, Jβ2.2, 5′-TAC GGT CAG CCT AGA GCC TTC-3′; Jβ2.3, 5′-CAC TGT CAG CCG GGT GCC TGG -5′; Jβ2.4, 5′-CAC TGA GAG CCG GGT CCC GGC-3′; Jβ2.5, 5′-CAC CAG GAG CCG CGT GCC TGG-3′; Jβ2.6, 5′-CAC GGT CAG CCT GCT GCC GGC-3′; Jβ2.7, 5′-GAC CGT GAG CCT GGT GCC CGG -3′. The sense Vβ primers were as follows: Vβ4, 5′-TGA GGC CAC ATA TGA GAG TGG-3′; Vβ7, 5′-GCT TCT CAC CTG AAT GCC CCA-3′; Vβ12, 5′-ACT CTG AGA TGT CAC CAG ACT-3′; Vβ13, 5′-ACA CTG CAG TGT GCC CAG GAT-3′.

RESULTS

Anti EBV-Abs, Surface Marker, and EBV Genome

The results of anti EBV-Ab titers, surface marker analysis, and the assessments of EBV genomes and TCR/Ig genes are shown in Table I. All patients showed abnormal high titers of EB VCA-IgG (≥1280), EADR-IgG (≥640) and the detection of VCA-IgA (≥10), and negative VCA-IgM and positive EBNA. CD3$^+$HLA-DR$^+$ cells increased, of which CD4$^+$ phenotype predominated in patients 1 and 2. On the other hand, patients 3 and 4 showed lower number of activated T-cells and lower CD4/CD8 ratio than patients 1 and 2.

EBV genome was assessed by the dot blotting PCR method probed with EBV-IR sequences, Southern blotting probed with EB-TR sequences, and/or in situ hybridization (ISH) using EBV-encoded RNA (EBER)1. EBV genomes were detected in all patients by the PCR-products method (data not shown). Southern blotting using TR sequence indicated the monoclonal nature (single band) of the EBV in patients 1, 3, and 4 (Table I). EBER1-ISH exhibited the high frequency of EBER$^+$ cells in tissue specimens in patients 2 (brain, spleen) and 4 (lymph node) (data not shown). When TCR or Ig gene rearrangements were assessed by Southern blotting of DNA samples obtained from the PMC using TCR Cβ, γ, Jβ1, 2 or JH, κ, λ gene probes, the rearranged bands of Cβ, γ, and Jβ2 genes were determined in patient 1, and those of Cβ gene were detected in patient 3 (Fig. 1). The germ line configuration was found in Ig genes studied.

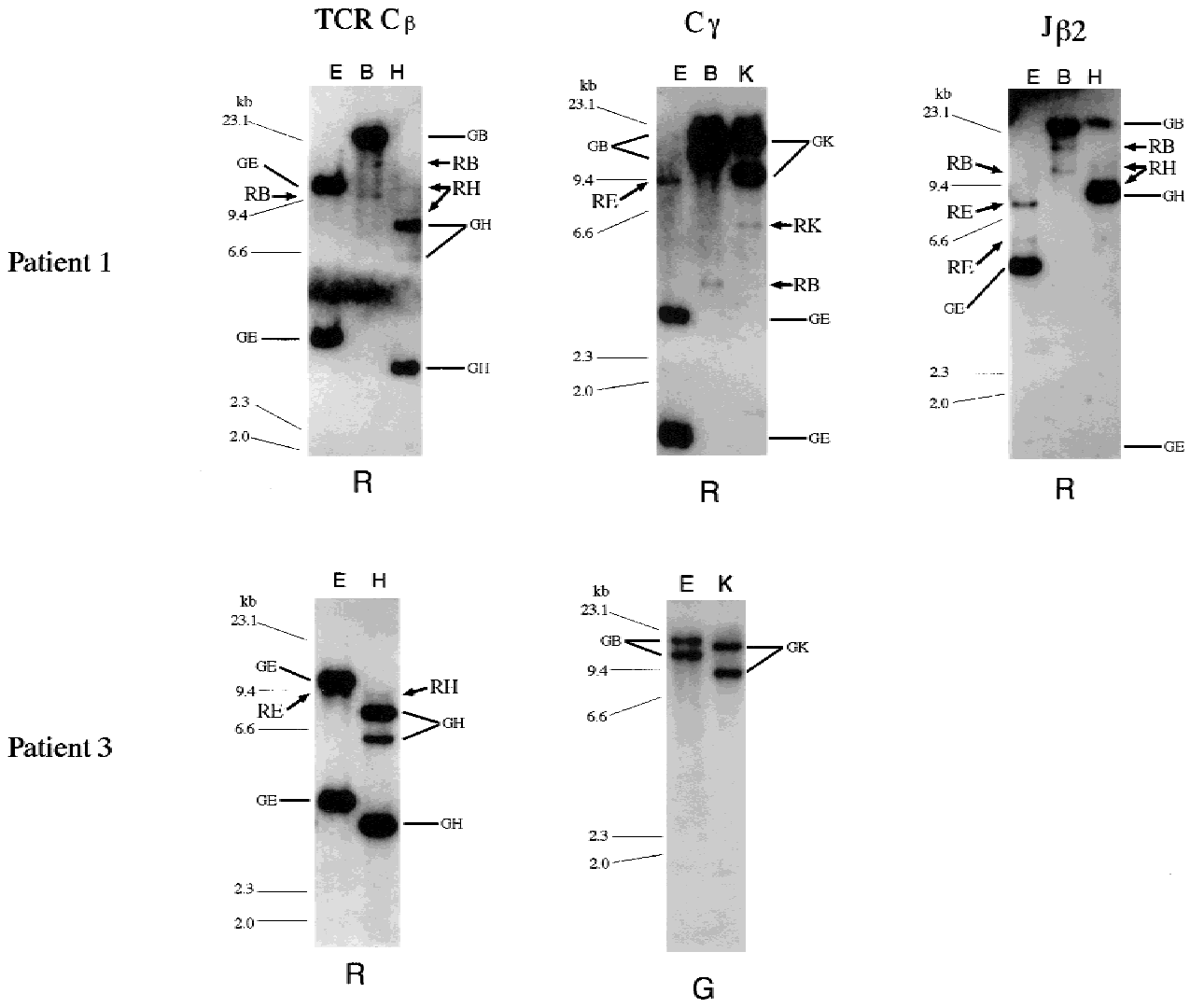

Fig. 1. TCR gene rearrangement patterns in patients 1 (upper) and 3 (lower) with CAEBV infection. Southern blot analysis of cellular DNA obtained from peripheral blood was performed with TCR Cβ, γ, Jβ2 gene probes as described previously [24]. E, *Eco*RI; B, *Bam*HI; H, *Hind* III; K, *Kpn*I; G, germ line band; R, rearrangement band.

TCR Vα and Vβ Gene Usages in Peripheral Lymphocytes

TCR Vα/βgene usages of lymphocytes obtained from these patients were examined by the inverse PCR methods (Fig. 2). In patient 1, Vα5 (49%) and Vα18 (35%) genes occupied more than 80% of total TCR Vα gene expression, and Vβ7 (29%) and Vβ12 (23%) genes were preferentially expressed. In patient 2, Vα1 (22%) and Vα18 (29%) genes attained to the half of total expression, and Vβ4 (32%) and Vβ13 (13%) genes were frequently expressed. In patient 3, no Vα gene exceeded 15% of total Vα gene expression, however, Vβ13 gene was remarkable at 28% of Vβ gene expression. In patient 4, Vα3 (22%) and Vβ13 (27%) genes were selectively used. Healthy controls did not show any specific clone which exceeded more than 15% of total Vα or Vβ gene expression.

Jβ Gene Expression in the Dominant Vβ Clones

To further investigate the clonality of T-cells bearing the specific Vβ gene, the Jβ gene usages of PCR products containing the specific Vβ gene were analyzed (Fig. 3). In patient 1, Jβ2.1 (25%) and Jβ2.7 (25%) fragments were prominent in Jβ-PCR products for Vβ7 gene. Jβ2.7 (35%) was also frequently expressed in Vβ12 clones. In patient 2, Jβ1.5 (81%) was exclusively used for Vβ4-PCR products. In patient 3, Jβ1.4 was expressed to 44% of the total expression of Vβ13 gene. In patient 4, Jβ1.5 occupied 78% of Vβ13 gene expressed. In summary, the skewing usages of Vβ gene and the preferential recombination of specific Jβ gene were outstanding in all CAEBV patients. Vβ4-Jβ1.5 and Vβ13-Jβ1.5 genes were clonally expressed in patients 2 and 4, respectively. The usage of the Vα18 gene was predominantly ob-

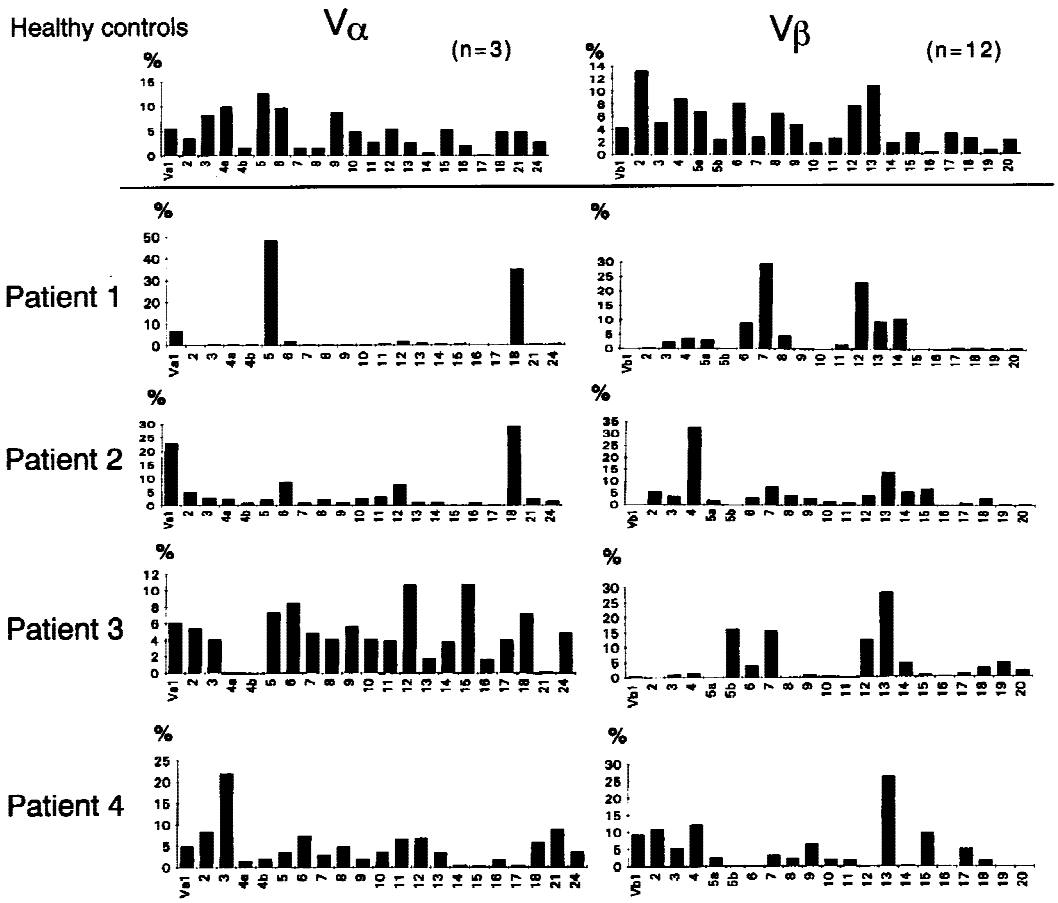

Fig. 2. TCR Vα or Vβ gene usages of peripheral lymphocytes by the inverse PCR method [23]. The double stranded cDNA was synthesized from total RNA obtained from peripheral blood of four patients. Following the circularization of the blunt-ended cDNA, PCR was separately performed using two constant region gene primers in opposite directions: Cα (forward and inverse) or Cβ (forward and inverse). The PCR mixture was electrophoresed on agarose, and the appropriate-sized cDNA fragments were extracted. The fragments were labeled with α-32P-dCTP and hybridized to the Vβ (from Vβ1 to Vβ20) or Vα fragments (from Vα1 to Vα18, Vα21, Vα24) dotted filters. These bar graphs depict the quantitative assessment of the V gene usages using densitometry.

served in patients 1 and 2, while the Vβ13 gene was selectively expressed in patients 3 and 4 (Table II).

DISCUSSION

The notable finding of the present study was the restricted diversity of TCR Vβ genes with the preferential Jβ gene expression in all CAEBV patients. TCR Vα gene usages were also restricted in 3 of 4 patients. This skewing TCR pattern was conspicuous compared with the variable diversification of healthy human TCR Vα, Vβ, and Jβ repertoire described previously [27], and that of our controls (Fig. 2). There was no report on the T-cell repertoire in persistent reactivation of EBV. Silins et al. [28] revealed the selection of a diverse TCR repertoire in response to anti-EBV encoded transactivator protein by $CD8^+$ CTL, and suggested that the biological role in maintaining a balanced viral load throughout EBV persistence. Smith et al. [29] reported the increased expression of Vβ6.1-3 and Vβ7 T-cells in acute IM, and proposed the superantigen response. Sutkowski et al. [30] have revealed the selective activation of human $Vβ13^+$ T-cells in response to EBV-infected cells, suggesting the role of EBV-associated superantigen in EBV infection. They further speculated that the symptoms of IM might be explained by the cytokine production and subsequent nonspecific T-cell activation via the superantigen-induced $Vβ13^+$ T-cells, and that the slower and weaker superantigen response in children was balanced by the induction of EBV-specific CTL to prevent clinical manifestation. In fact, the preferential expansion of Vβ13 gene was observed in patients 3 and 4; however, the Jβ gene usages indicated oligo- or mono-clonal expansion of $Vβ13^+$ cells (Fig. 3). Furthermore, Jβ2.7 gene expression in Vβ7 and Vβ12 clones in patient 1 well reflected the Cβ and Jβ gene rearrangements assessed by Southern

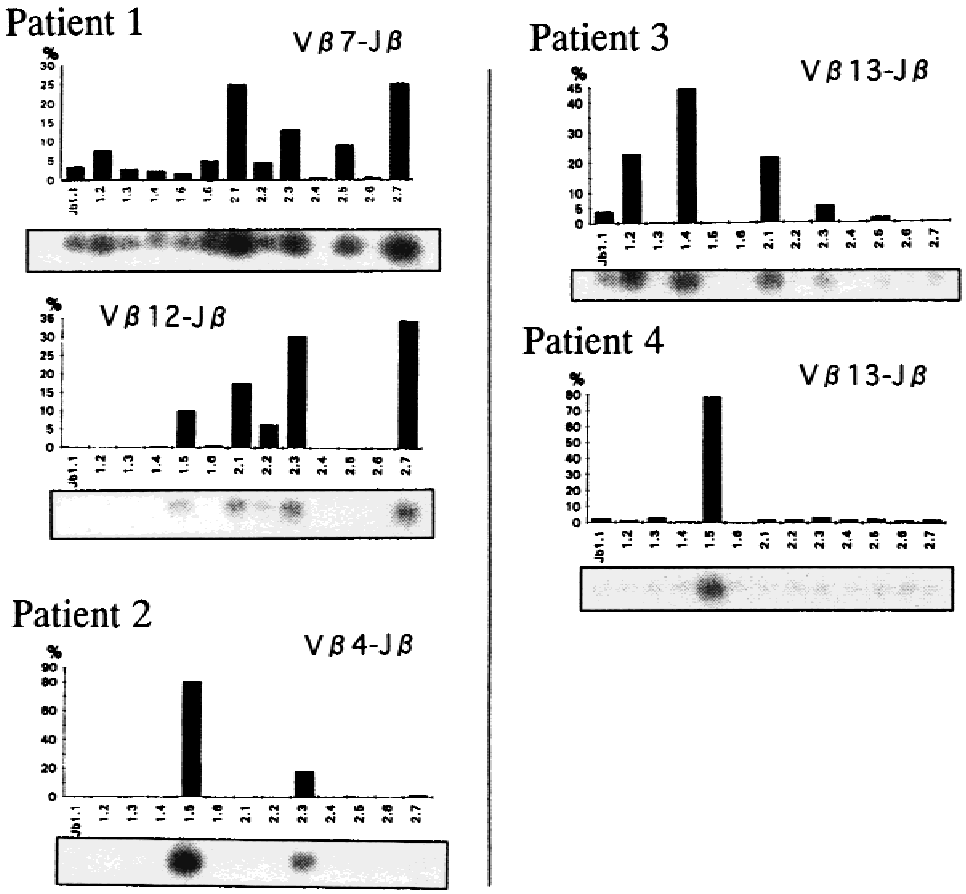

Fig. 3. Jβ gene usages in the predominated Vβ clones. When a certain Vβ gene was preferentially used, RT-PCR amplification was carried out using the sense Vβ gene primer and a total of 13 sets of antisense Jβ primers. These bar-graphs illustrate the ratio of each hybridized intensity of Jβ gene products to the total amounts of hybridization. Each figure below the graph shows the real intensity of hybridization.

TABLE II. T-cell Repertoire in Patients With Chronic Active Epstein-Barr Virus Infection

Patient	Complication	TCR gene rearrangement[a]	TCR V or J gene usages[b]		
			Vα	Vβ	Vβ/Jβ
1	Severe	Cβ,Jβ2,Cγ	5,18	7,12	7/2.7, 12/2.7
2	Severe	No	1,18	4	4/1.5
3	Nonsevere	Cβ	Variable	13	13/1.4
4	Nonsevere	No	3	13	13/1.5

[a]Southern blotting.
[b]Inverse PCR method.

blotting (Fig. 1). Callen et al. [31] have revealed recently that large monoclonal or oligoclonal populations of $CD8^+$ T-cells expanded in acute phase of IM, and disappeared in convalescent phase. Sequence analysis of CDR3 and its corresponding Jβ gene selection strongly indicated that these populations had been driven by viral antigens. In this context, our data support that the sustained expansion of T-cells was clonally driven by conventional viral antigens rather than superantigens. Human TCR V-segment frequencies in peripheral blood are influenced profoundly by the HLA complex [32]. Argaet et al. [33] described the dominant selection of an invariant TCR from HLA-B8-restricted $CD8^+$ cytotoxic T-cell clones in response to persistent infection by EBV. The disparate TCR diversity in each patient may partly account for the differences in the HLA complex and/or the proportion of $CD8^+$ T-cells.

Imai et al. [17] established four T-cell lines infected with EBV, one of which expressed $CD4^+$ $CD8^-$ $CD45^+$ phenotype accompanied by clonal TCR β and γ gene rearrangements. Both rearrangements of TCR β and γ genes were also determined in patient 1. Ishihara et al. [22] described clonal lymphoproliferation in CAEBV patients, two of whom showed the peripheral increase of $CD4^+$ T-cells. In patients 1 and 2, the increase of activated $CD4^+$ $CD8^-$ T-cells correlated with the disease progression. Histopathological analysis of the brain lesion in patient 2 revealed that the major infiltration was composed of EBV-infected activated $CD4^+$ T-cells (data not shown). Paterson et al. [34,35] indicated that human immature thymocyte was activated after EBV infection

and its consequences at an early stage of differentiation might lead to failure of normal T-cell repertoire development. Taken together, the clonal expansion of EBV-infected T-cells may affect the derangement of T-cell diversity and contribute to lymphoproliferation in CAEBV patients.

From another point of view, the reduced fractions of each Vα, Vβ T-cell may be of much more importance than the expanded subpopulations. The engrafted hematopoietic cells can occasionally express a restricted clonal diversity of T-cells in severe combined immunodeficiency [36]. The clonal expansion and exhaustion of T-cells might also occur in patients with human immunodeficiency virus infection. Patients with X-linked lymphoproliferative disease (XLP) succeed to the defective cellular immunity to EBV and develop LPD/lymphomas as a consequence. In the present study, clonal T-cell proliferation was not always associated with the clinical severity and the increased number of activated T cells (Table II). It may raise speculation that CAEBV-T cells carry an indigenous defect to organize the balanced T-cell repertoire during persistent EBV infection. Further fractionation analyses on EBV-infected T-cells are required not only for understanding the pathogenesis of CAEBV, but also for characterizing the clinical entity of primary T-cell deficiency disease triggered by EBV infection.

ACKNOWLEDGMENTS

We are grateful to Takaaki Kanamaru, MT (Morphology Core, Faculty of Medicine, Kyushu University, Fukuoka, Japan) for technical assistance, and Brian T. Quinn (Associate Professor, Kyushu University, Fukuoka, Japan) for reviewing this manuscript. This work was supported in part by a grant from the Ministry of Health and Welfare for Primary Immunodeficiency Diseases, and by a grant-in-aid for the encouragement of young scientists from the Ministry of Education, Science and Culture of Japan.

REFERENCES

1. Jones JF. A perspective of Epstein-Barr virus diseases. Adv Pediatr 1989;36:307–346.
2. Pallesen G, Hamilton-Dutoit SJ, Zou X. The association of Epstein-Barr virus (EBV) with T cell lymphoproliferations and Hodgkin's disease: Two new developments in the EBV field. Adv Cancer Res 1993;62:179–239.
3. Harabuchi Y, Imai S, Wakashima J, Hirao M, Kataura A, Osato T, Kon S. Nasal T-cell lymphoma casually associated with Epstein-Barr virus. Cancer 1996;77:2137–2149.
4. Sasajima Y, Yamabe H, Kobashi Y, Hirai K, Mori S. High expression of the Epstein-Barr virus latent protein EB nuclear antigen-2 on pyothorax-associated lymphomas. Am J Pathol 1993;143:1280–1285.
5. Imai S, Koizumi S, Sugiura M, Tokunaga M, Uemura Y, Yamamoto N, Tanaka S, Sato E, Osato T. Gastric carcinoma: monoclonal epithelial malignant cell expressing Epstein-Barr virus latent infection protein. Proc Natl Acad Sci USA 1994;91:9131–9135.
6. Leyvraz S, Henle W, Chahinian AP, Perlmann C, Klein G, Gordon RE, Rosenblum M, Holland JF. Association of Epstein-Barr virus with thymic carcinoma. N Engl J Med 1985;312:1296–1299.
7. Elenitoba-Johnson KSJ, Jaffe ES. Lymphoproliferative disorders associated with congenital immunodeficiencies. Semin Diagn Pathol 1997;14:35–47.
8. Riddler SA, Breinig MC, McKnight JLC. Increased levels of circulating Epstein-Barr virus (EBV)-infected lymphocytes and decreased EBV nuclear antigen antibody response are associated with the development of posttransplant lymphoproliferative disease in solid-organ transplant recipients. Blood 1994;84:972–984.
9. Shearer WT, Ritz J, Finegold MJ, Guerra IG, Rosenblatt HM, Lewis DE, Pollack MS, Taber LH, Sumaya CV, Grumet FC. Epstein-Barr virus associated B-cell proliferations of diverse clonal origins after bone marrow transplantation in a 12-year-old patient with severe combined immunodeficiency. N Engl J Med 1985;312:1151–1159.
10. Mustafa MM, Winick NJ, Margraf, LR. Epstein-Barr virus lymphoproliferative disorder in children with leukemia: case report and review of the literature. J Pediatr Hematol/Oncol 1997;19:77–81.
11. Rickinson AB. Chronic, symptomatic Epstein-Barr virus infections. Immunol Today 1986;7:13–14.
12. Straus SE. The chronic mononucleosis syndrome. J Infect Dis 1988; 157:405–412.
13. Okano M, Matsumoto S, Osato T, Sakiyama Y, Thiele GM, Purtilo DT. Severe chronic active Epstein-Barr virus infection syndrome. Clin Microbiol Rev 1991;4:129–135.
14. Ishihara S, Okada S, Wakiguchi H, Kurashige T, Morishima T, Kawa-Ha K. Chronic active Epstein-Barr virus infection in children in Japan. Acta Paediatr 1995;84:1271–1275.
15. Tosato G, Straus S, Henle W, Pike SE, Blaese RM. Characteristic T cell dysfunction in patients with chronic active Epstein-Barr virus infection (chronic infectious mononucleosis). J Immunol 1985;134: 3082–3088.
16. Fujieda M, Wakiguchi H, Hisakawa H, Kubota H, Kurashige T. Defective activity of Epstein-Barr virus (EBV) specific cytotoxic T lymphocytes in children with chronic active EBV infection and in their parents. Acta Paediatr Jpn 1993;35:394–399.
17. Imai S, Sugiura M, Oikawa O, Koizumi S, Hirao M, Kimura H, Hayashibara H, Terai N, Tsutsumi H, Oda T, Chiba S, Osato T. Epstein-Barr virus (EBV)-carrying and -expressing T-cell lines established from severe chronic active EBV infection. Blood 1996;87:1446–1457.
18. Ishihara S, Tawa A, Yumura-Yagi K, Murata M, Hara J, Yabuuchi H, Hirai K, Kawa-Ha K. Clonal T-cell lymphoproliferation containing Epstein (EB) virus DNA in a patient with chronic active EB virus infection. Jpn J Cancer Res 1989;80:99–101.
19. Ohga S, Matsuzaki A, Tokunaga K, Yamamori S, Suda M, Fujisawa K, Inomata H, Ishii E, Ueda K. Clonal dissemination of Epstein-Barr (EB) virus in a patient with fatal chronic active EBV infection. Int J Pediatr Hematol/Oncol 1994;1:305–310.
20. Kikuta H, Taguchi Y, Tomizawa K, Kojima K, Kawamura N, Ishizuka A, Sakiyama Y, Matsumoto S, Imai S, Kinoshita T. Epstein-Barr virus genome-positive T lymphocytes in a boy with chronic active EBV infection associated with Kawasaki-like disease. Nature 1988;333: 455–457.
21. Kikuta H, Sakiyama Y, Matsumoto S, Hamada I, Yazaki M, Iwaki T, Nakano M. Detection of Epstein-Barr virus DNA in cardiac and aortic tissues from chronic, active Epstein-Barr virus infection associated with Kawasaki disease-like coronary artery aneurysm. J Pediatr 1993; 123:90–92.
22. Ishihara S, Okada S, Wakiguchi H, Kurashige T, Hirai K, Kawa-Ha K. Clonal lymphoproliferation following chronic active Epstein-Barr virus infection and hypersensitivity to mosquito bites. Am J Hematol 1997;54:276–281.
23. Kimura N, Yoshida T, Kumagawa M, Akiyoshi T, Nagano M, Yuh K,

Kikuchi M, Okumura M. Analysis of the T-cell receptor (TCR) repertoire due to the newly devised inverse polymerase chain reaction (PCR) method. 8th Congress of Asian Pacific Division International Society of Haematology 149 [abstract]. 1995.

24. Sakai Y, Ohga S, Tonegawa Y, Takada H, Nakao F, Aoki T, Yamamori S, Hara T: Interferon-α therapy for chronic active Epstein-Barr virus infection: potential effect of the development of T-lymphoproliferative disease. J Pediatr Hematol/Oncol 1998;20:342–346.
25. Ohga S, Yoshikai Y, Takeda Y, Hiromatsu K, Nomoto K. Sequential appearance of γ/δ- and α/β-bearing T cells in the peritoneal cavity during an intraperitneal infection with *Listeria monocytogenes.* Eur J Immunol 1990;20:533–538.
26. Kimura N, Toyonaga B, Yoshikai Y, Du RP, Mak TM. Sequences and repertoire of the human T cell receptor α and β chain variable region genes in thymocytes. Eur J Immunol 1987;17:375–383.
27. Rosenberg WMC, Moss PAH, Bell JI. Variation in human T cell receptor Vβ and Jβ repertoire: analysis using anchor polymerase chain reaction. Eur J Immunol 1992;22:541–549.
28. Silins SL, Cross SM, Elliott SL, Pye SJ, Burrows JM, Moss DJ, Misko IS. Selection of a diverse TCR repertoire in response to an Epstein-Barr virus-encoded transactivator protein BZLF1 by $CD8^+$ cytotoxic T lymphocytes during primary and persistent infection. Int Immunol 1997;9:1745–1755.
29. Smith TJ, Terada N, Robinson CC, Gelfand EW. Acute infectious mononucleosis stimulates the selective expression/expansion of Vβ6.1-2 and Vβ7 T-cells. Blood 1993;81:1521–1526.
30. Sutkowski N, Palkama T, Ciurli C, Sekaly R-P, Thorley-Lawson DA, Huber BT. An Epstein-Barr virus-associated superantigen. J Exp Med 1996;184:971–980.
31. Callen MFC, Steven N, Krausa P, Wilson JDK, Moss PAH, Gillespie GM, Bell JI, Rickinson AB, McMichael AJ. Large clonal expansions of $CD8^+$ T-cells in acute infectious mononucleosis. Nature Med 1996; 2:906–911.
32. Gulwani-Akolkar B, Posnett DN, Janson CH, Grunewald J, Wigzell H, Akolkar P, Gregersen PK, Silver J. T cell receptor V-segment frequencies in peripheral blood T cells correlate with human leukocyte antigen type. J Exp Med 1991;174:1139–1146.
33. Argaet VP, Schmidt CW, Burrows SR, Silins SL, Kurilla MG, Doolan DL, Suhrbier A, Moss DJ, Kieff E, Sculley TB, Misko IS. Dominant selection of an invariant T cell antigen receptor in response to persistent infection by Epstein-Barr virus. J Exp Med 1994;180:2335–2340.
34. Paterson RLK, Kelleher CA, Streib JE, Amonkonah TD, Xu JW, Jones JF, Gelfand EW. Activation of human thymocytes after infection by EBV. J Immunol 1995;154:1440–1449.
35. Paterson RLK, Kelleher C, Amankonah TD, Streib JE, Xu JW, Jones JF, Gelfand EW. Model of Epstein-Barr virus infection of human thymocytes: expression of viral genome and impact on cellular receptor expression in the T-lymphoblastic cell line, HPB-ALL. Blood 1995;85:456–464.
36. Sottini A, Q-Roldan E, Notarrangelo LD, Malagoli A, Primi D, Imberti L. Engrafted maternal T cells in a severe combined immunodeficiency patient express T-cell receptor variable beta segments characterized by a restricted V-D-J junctional diversity. Blood 1995;85:2105–2113.